

### Figure 1:

# Delta-6 Desaturase Sequence Alignment:

Consensus key (see documentation for details)

- \* single, fully conserved residue
  - conservation of strong groupsconservation of weak groups
- no consensus

CLUSTAL W (1.81) multiple sequence alignment

Mucor-D6	
Rhizopus-D6	
Malpina-D6	
Sdiclina-D6	
Phytium-D6	
Phaeo-D6	<u> </u>
Physcomit-D6	MVFAGGGLQQGSLEENIDVEHIASMSLFSDFFSYVSSTVGSWSVHSIQPLKRLTSKKRVS
Ceratodon-D6	MVSQGGGLSQGSIEENIDVEHLATMPLVSDFLNVLGTTLGQWSLSTTFAFKRLTTKKHSS
C.elegansD6	
Borage-D6	
Human-D6	
Mucor-D6	L-DKKPEARK
Rhizopus-D6	1 1 1 1
Malpina-D6	EGKKDAEAPF

Sdiclina-D6 Phytium-D6 Phaeo-D6 Physcomit-D6 Ceratodon-D6 C.elegansD6 Borage-D6 Human-D6	
Mucor-D6 Rhizopus-D6 Malpina-D6 Sdiclina-D6 Phytium-D6 Physcomit-D6 Ceratodon-D6 C.elegansD6 Borage-D6	LIVVENKVYDITDFVFDHPGGERVLLTQEGRDATDVFHEMHPPSAYELLANCYVGDCE FIIIDRKVYDVTEFLEDHPGGAQVLLTHVGKDASDVFHAMHPESAYELLANCYVGDVK LMIIDNKVYDVTEFLEDHPGGS-VILTHVGKDGTDVFHAMHPESAWETLANFYVGDID WIVIHHKVYDISAFE-DHPGGV-VMFTQAGEDATDAFAVFHPSSALKLLEQYYVGDVD WIVIHHKVYDISKWD-SHPGGS-VMLTQAGEDATDAFAVFHPSSALKLLEQFYVGDVD WIIHSNKVYDVSNWH-EHPGGA-VIFTHAGDDMTDIFAAFHAPG-SQSLMKKFYIGELL WIVVKNKVYDVSNFADEHPGGS-VISTYFGRDGTDVFSSFHAASTWKILQNFYIGDVF WIIIKEKVYDVSTFAEQHPGGT-VINTYFGRDATDVFSTFHASTSWKILQNFYIGNLV MKVDGKWLYLSEELVKKHPGGA-VIEQYRNSDATHIFHAFHEGSSQAYKQLDLLKKHGEH WISIQGKAYDVSDWVKDHPGGSFPLKSLAGQEVTDAFVAFHPASTWKNLDKFFTGYYL WLVIDRKVYNITKWSIQHPGGORVIGHYAGEDATDAFRAFHPDLEFVGKFLKPLLIGELA

-----ESDRAIKND------DFAAEVRKLRTLFQSLGYYDSSKAYYAFKV -----DAHVKETPSA-------QFASEMRQLRDQLKKEGYFHSSKAYYVYKV ----QSTAAVDTS--ISDEVKKSQ---SDFIASYRKLRLEVKRLGLYDSSKLYYLYKC ---AFAQEIRDLRDKLEKQGYFDASTGFYIYKV PKLPIDSTDKKALNSA----Rhizopus-D6 Sdiclina-D6

Malpina-D6

Mucor-D6

Phytium-D6	ETSKAEIEGEPASDEERARRERINEFIA
Phaeo-D6	PETTGKEPQQIAFEK(
Physcomit-D6	
Ceratodon-D6	ELLK
C.elegansD6	DEFLEKQLEKRLDKVDINVSAYDVSVAQEKKMVE:
Borage-D6	KDYSVSEVSKI
Human-D6	PEEPSQDHGKNSKITE

SYRRLRVKVKGMGLYDASALYYAWKL GYRDLRSKLIMMGMFKSNKWFYVYKC EYRELRALFLREQLFKSSKSYYLFKT SFEKLRQKLHDDGLMKANETYFLFKA DYRKLVFEFSKMGLYDKKGHIMFATL

DFRALRKTAEDMNLFKTNHVFFLLLL

**DFREMRALFLREQLFKSSKLYYVMKL** 

ASTLSIALVSAAICLHFD--STAMYMVAAVILGLFYQQCGWLAHDFLHHQVFENHLFGDL VSTFGIAVLSMAICFFFN--SFAMYMVAGVIMGLFYQQSGWLAHDFLHNQVCENRTLGNL LSNMAIWAAACALVFYSD--RFWVHLASAVMLGTFFQQSGWLAHDFLHHQVFTKRKHGDL LTNVAIFAASIAIICWSK--TISAVLASACMMALCFQQCGWLSHDFLHNQVFETRWLNEV LINVSIVATSIAIISLYK--SYRAVLLSASLMGLFIQQCGWLSHDFLHHQVFETRWLNDV ISTLSIMAFAFYLQYLG----WYITSACLLALAWQQFGWLTHEFCHQQPTKNRPLNDT CFIAMLFAMSVYGVLFCEG--VLVHLFSGCLMGFLWIQSGWIGHDAGHYMVVSDSRLNKF STTLLVCIVGLAILKAWGRESTLAVFIAASLVGLFWQQCGWLAHDYAHYQVIKDPNVNNL SFNLCIWGLSTFIVAKWGQTSTLANVLSAALLGLFWQQCGWLAHDFLHHQVFQDRFWGDL LSTLALCAAGLTLLYAYGHTSTLAVVASAIIVGIFWQQCGWLAHDFGHHQCFEDRSWNDV AHIIALESIAWFTVFYFGN-GWIPTLITAFVLATSQAQAGWLQHDYGHLSVYRKPKWNHL

> Physcomit-D6 Ceratodon-D6

C.elegansD6

Borage-D6

Human-D6

Sdiclina-D6

Phytium-D6

Phaeo-D6

Malpina-D6

Rhizopus-D6

Mucor-D6

Mucor-D6 Rhizopus-D6 Malpina-D6 **Sdiclina-D6** Phytium-D6

-DPDIDTAPILLWDEFAVANF ---DPDIDTHPLLTWSEHALEMF ---DPDIDTAPVLLWDEYASAAY [GCLVGNAWQGFSVQWWKNKHNLHHAVPNLHSAKDEGFIGDPDIDTMPLLAWSKEMAR--VGVMVGNLWQGFSVQWWKNKHNTHHAIPNLHATPEIAFHGDPDIDTMPILAWSLKMAQ--LVVFLGNFCQGFSLSWWKNKHNTHHASTNVHGH--FGAFLGGVCQGFSSSWWKDKHNTHHAAPNVHGE-FLVTFGNLVQGFSLSWWKNKHNTHHASTNVSGE-

aeo-D6	yscomit-D6	eratodon-D6	elegansD6	Borage-D6	Human-D6
Pha	Phy	Cer	e	30r	Hum

MGIFAANCLSGISIGWWKWNHNAHHIACNSLEY-----DPDLQYIPFLVVSSKFFGSL ----DPDVNMLHVFVLGEWQPIEY VGYVVGNVVLGFSVSWWKTKHNLHHAAPNECDQKYTP--IDEDIDTLPIIAWSKDLLAT-ISLFFGNFLQGFSRDWWKDKHNTHHAATNVIDH-----DGDIDLAPLFAFIPGDLCKY VGYVIGNAVLGFSTGWWKEKHNLHHAAPNECDQTYQP--IDEDIDTLPLIAWSKDILAT-3GLFWGNLMQGYSVQWWKNKHNGHHAVPNLHCSSAVAQDGDPDIDTMPLLAWSVQQAQSY VHKFVIGHLKGASANWWNHRHFQHHAKPNIFHK--

Mucor-D6
Rhizopus-D6
Malpina-D6
Sdiclina-D6
Phytium-D6
Phaeo-D6
Physcomit-D6
Ceratodon-D6
C.elegansD6
Human-D6

RELQADG---KDSGLVKFMIRNQSYFYFPILLLARLSWLNESFKCAFGLGAASENAALEL -----VENKTFLRILQYQHLFFMGLLFFARGSWLFWSWRYTST----AVLSPVD -----VESKTMLRVLQYQHLFFLVLLTFARASWLFWSAAFTLR-----PELTLGE YASLDEEPTMISRFLAESVLPHQTRYYFFVLGFARLSWAIQSLLYSFK-----QGAINKS SDVPDEE---LTRMWSRFMVLNQTWFYFPILSFARLSWCLQSIMFVLP----NGQAHKP ---YNVGPGG YGSLKDNASGFDRFIAEHILPYQTRYYFFILGFARTSWAIQSIIYSFK----NETLNKS ----KA---FESAHGPFFIRNQAFLYFPLLLLARLSWLAQSFFYVF----TEFSFG-KAS-----FEKAILKIVPYQHLYFTAMLPMLRFSWTGQSVQWVFK---TSHFYEKR-LTFDSLSRFFVSYQHWTFYPIMCAARLNMYVQSLIMLLT-------KKKLKYLPYNHQHEYFFLIGPPLLIPMYFQYQIIMTM------HA---VDSPVGLFFMRYQAYLYFPILLFARISWVIQSAMYAF--

> Mucor-D6 Rhizopus-D6 Malpina-D6 **Sdiclina-D6** Phytium-D6 Phaeo-D6

K----LLSWCERIFLIVHWVFFTYCTIAWIS-SIRNIAMFFVVSQITTGYLLAIVFAMNH H----QINLFERFCLVSHWTLFTYCTLAWCS-NVYHMILFFLVSQATTGYTLALVFALNH SGARVPISLVEQLSLAMHWTWYLATMFLFIK-DPVNMIVYFLVSQAVCGNLLAIVFSLNH TFDKVQYPLLERAGLLLYYGWNLGLVY-AANMSLLQAAAFLFVSQASCGLFLAMVFSVGH IFDKVEFDGPEKAGLIVHYIWQLAIPY-FCNMSLFEGVAYFLMGQASCGLLLALVFSIGH KAKGLQYPLLEKAGILLHYAWMLTVSSGFGRFSFAYTAFYFLTATASCGFLLAIVFGLGH

yscomit-D6	ratodon-D6	elegansD6	orage-D6	Human-D6
Phys	Cera	(a)	30rë	um

R-----LLEKGTVLFHYFWFVGTACYLLP--GWKPLVWMAVTELMSGMLLGFVFVLSH K-----LLERGTMALHYIWFNSVAFYLLP--GWKPVVWMVVSELMSGFLLGYVFVLSH YKVYQRNAFWEQATIVGHWAWVFYQLFLLPT--WPLRVAYFIISQMGGGLLIAHVVTFNH -KRNVSYRAHELLGCLVFSIWYPLLVSCLPN---WGERIMFVIASLSVTGMQQVQFSLNH ---IVHKNWVDLAWAVSYYIRFFITYIPFYG--ILGALLFLNFIRFLESHWFVWVTQMNH

Mucor-D6
Rhizopus-D6
Malpina-D6
Sdiclina-D6
Phytium-D6
Physcomit-D6
Ceratodon-D6
C.elegansD6
Human-D6

NGMATYNAD--ARPDFWKLQVTTTRNVTGGHGFPQAFVDWFCGGLQYQVDHHLFPSLPRH

NGMEVYN----SSKEFVSAQIVSTRDIK----GNIFNDWFTGGLNRQIEHHLFPTMPRH

NSVDKYPANSRILNNFAALQILTTRNMT----PSPFIDWLWGGLNYQIEHHLFPTMPRC

NGMEVYN----TSKDFVNAQIASTRDIK---

NGMEVFDKD--SKPDFWKLQVLSTRNVT----SSLWIDWFMGGLNYQIDHHLFPMVPRH NGMSVYERE--TKPDFWQLQVTTTRNIR----ASVFMDWFTGGLNYQIDHHLFPLVPRH

NGMPVISKEEAVDMDFFTKQIITGRDVH---

NGMPVYSPEEANHTEFYELQCITGRDVN-----CTVFGDWLMGGLNYQIEHHLFPEMPRH NGMPVITEEKAESMEFFEIQVITGRDVT----LSPLGDWFMGGLNYQIEHHVFPNMPRH

--PGLFANWFTGGLNYQIEHHLFPSMPRH

--AGVFNDWFTGGLNRQIEHHLFPTMPRH

FSSSVYVGK-PKGNNWFEKQTDGTLDIS----CPPWMDWFHGGLQFQIEHHLFPKMPRC IVMEIDQEA---YRDWFSSQLTATCNVE----QSFFNDWFSGHLNFQIEHHLFPTMPRH \*\*\* \*\*\*\*\* \* \* \* \* \* \* \* \*

Mucor-D6
Rhizopus-D6
Malpina-D6
Sdiclina-D6
Phytium-D6
Phaeo-D6
Physcomit-D6

NLPKVKPMVKSLCKKYDINYHDTGFLKGTLEVLKTLDITS----KLSLQLSKKSF---NFSKIQPAVETLCKKYGVRYHTTGMIEGTAEVFSRLNEVS----KAASKMGKAQ----NLPALNVLVKSLCKQYDIPYHETGFIAGMAEVVVHLERIS----IEFFKEFPAM----NLPKVNVLIKSLCKEFDIPFHETGFWEGIYEVVDHLADIS----KEFITEFPAM---NLAKTHALVESFCKEWGVQYHEADLVDGTMEVLHHLGSVAGEFVVDFVRDGPAM--NLNKIAPRVEVFCKKHGLVYEDVSIATGTCKVLKALKEVAE---AAAEQHATTS----KISQKFSKKML-HLSKVKSMVKPIAQKYNIPYHDTTVIGGTIEVLQTLDFVQ--

Ceratodon-D6 NLNKISPHVETLCKKHGLVYEDVSMASGTYRVLKTLKDVADAASHQQLAASC.elegansD6 NLNACVKYVKEWCKENNLPYLVDDYFDGYAMNLQQLKNMAEHIQAKAABorage-D6 NLRKISPYVIELCKKHNLPYNYASFSKANEMTLRTLRNTALQARDITKPLPKNLVWEALHHuman-D6 NLHKIAPLVKSLCAKHGIEYQEKPLLRALLDIIRSLKKSGKLWLDAYLHKNLE	1 1	Rhizopus-D6	Malpina-D6	Sdiclina-D6	Phytium-D6	!!	Physcomit-D6	Ceratodon-D6	C.elegansD6	THG	
KTLKDVADAASHQQLAAS QQLKNMAEHIQAKAA RTLRNTALQARDITKPLPKNLVWEALH RSLKKSGKLWLDAYLHKNLE											



#### Figure 2:

versus Pythium Comparison of nucleotide sequence of S. diclina  $\Delta 6$ -desaturase irregulare  $\Delta 6$ -desaturase:

Average Match: 10.000 Average Mismatch: 0.000 50 Gap Weight: Length Weight:

1383 Length: 9148 Quality: Percent Identity: 68.948 Percent Similarity: 68.948

6.717

Ratio:

Match display thresholds for the alignment(s):

= IDENTITY

## S. diclina-D6 x Pythium irregulare-D6

- 145 ACGCAGGCCGGCGAAGACGCGACCGATGCGTTCGCTGTCTTCCACCCGAG 194

629	
6	
3AC	
$\mathcal{L}$	Ξ
$\mathbf{z}$	_
$\vec{c}$	-
K	-
Š	_
$\mathcal{L}$	Ξ
$\supset$	_
$\ddot{c}$	_
Ā	-
Æ	-
9	-
$\Rightarrow$	_
3	_
Ö	_
Ē	_
Ö	-
9	-
5	Ξ
Æ	_
$\ddot{c}$	_
Ũ	_
H	-
Ö	-
Ж	
ĭ	
Ü	_
₽	-
H	-
Ж	_
$\mathcal{C}_{\mathcal{C}}$	_
Ö	_
K	-
$\ddot{\circ}$	-
õ	-
2	Ξ
5	_
ACCTCTGGCAGGGCTTCTCGGTGCAGTGGTGGAAGAACAAGCACAAC	
Ü	
Q	-
5	_
7	_
0	
œ	

- 601 AACGCCTGGCAGGGCTTCAGCGTGGTGGAAGAACAAGCACAACCT 650
- 630 GCACCATGCGATCCCCAACCTCCACGCGCCCGAGATCGCCTTCCACG 679
  - 651 GCACCACGCGGTGCCGAACCTGCACAGCGCCAAGGACGAGGGCTTCATCG 700
- 680 GCGACCCGGACATTGACACGATGCCGATTCTCGCGTGGTCGCTCAAGATG 729
  - 701 GCGACCCGGACATCGACACCATGCCGCTGCTGGCGTGGTCTAAGGAGATG 750
- 730 GCGCAGCACGCGGTCGACTCGCCCGTCGGGCTCTTCTTCATGCGCTACCA 779
- . 780 AGCGTACCTGTACTTTCCCATCTTGCTCTTTGCGCGTATCTCGTGGGTGA 829
  - 801 GGCGTTCCTATACTTCCCGCTGCTGCTGCTCGCGCGCCTGAGCTGGCTCG 850
- 830 rccagrcgccargracgccrrcracaacgrrgggcccggcgcaccrrr 879
- 851 CGCAGTCGTTCTTCTACG...TGTTCACCGAGTTCTCGTTCGGCATCTTC 897
- 880 GACAAGGICCAGIACCCGCIGCICGAGCGCGCCGCCCTCCICCICTACIA 929
- 898 GACAAGGTCGAGTTCGACGGACCGGAGAGGCGGGTCTGATCGTGCACTA 947
- 930 CGGCTGGAACCTCGGCCTTGTACGCAGCCAACATGTCGCTGCTCCAAG 979
  - 948 CATCTGGCAGCTCGCGATCCCGTACTTCTGCAACATGAGCCTGTTTGAGG 997
- 980 CGCTGCGTTCCTCTTTGTGAGCCAGGCGTCGTGCGGCCTCTTCCTCGCG 1029



#### Figure 3:

Comparison of amino acid sequence of  $\Delta 6$ -desaturases from S. diclina and P. irregulare

S. diclina  $\Delta 6$ -desaturase Query= Pythium Irrequlare  $\Delta 6$ -desaturase Subject:

Pythium irregulare delta-6 fatty acid desaturase >gi|16033736|gb|AF419296.1|AF419296

mRNA, complete cds Length = 1380 Score = 548 bits (1412), Expect = e-154

Identities = 267/449 (59%), Positives = 328/449 (73%), Gaps = 5/449 (1%) Frame = +1

69 ISWATIREHNRQDNAWIVIHHKVYDISAFEDHPGGVVMFTQAGEDATDAFAVFHPSSALK Query: 10

AWIVIHHKVYDIS ++ HPGG VM TQAGEDATDAFAVFHPSSALK

213 VSWKEIREHATPATAWIVIHHKVYDISKWDSHPGGSVMLTQAGEDATDAFAVFHPSSALK Sbjct: 34

LLEQYYVGDVDQSTAAV--DTSISDEVKKSQ---SDFIASYRKLRLEVKRXXXXXXXXXX 124 Query: 70

LLEQFYVGDVDETSKAEIEGEPASDEERARRERINEFIASYRRLRVKVKGMGLYDASALY ++FIASYR+LR++VK LLEQ+YVGDVD+++ A

Sbjct: 214

Query: 125 XXXXCASTLSIALVSAAICLHFDSTAMYMVAAVILGLFYQQCGWLAHDFLHHQVFENHLF 184

ST IA++S AIC F+S AMYMVA VI+GLFYQQ GWLAHDFLH+QV EN

573 Sbjct: 394 YAWKLVSTFGIAVLSMAICFFFNSFAMYMVAGVIMGLFYQQSGWLAHDFLHNQVCENRTL

- Query: 185 GDLVGVMVGNLWQGFSVQWWKNKHNTHHAIPNLHATPEIAFHGDPDIDTMPILAWSLKMA
  - 753 GNLIGCLVGNAWQGFSVQWWKNKHNLHHAVPNLHSAKDEGFIGDPDIDTMPLLAWSKEMA F GDPDIDTMP+LAWS +MA + G+L+G +VGN WQGFSVQWWKNKHN HHA+PNLH+ 574 Sbjct:
- 304 245 QHAVDSPVGLFFMRYQAYLYFPILLFARISWVIQSAMYAFYNVGPGGTFDKVQYPLLERA Query:
- G FF+R QA+LYFP+LL AR+SW+ QS Y F + A +S
- 930 Sbjct: 754 RKAFESAHGPFFIRNQAFLYFPLLLLARLSWLAQSFFYVFTEFS-FGIFDKVEFDGPEKA
- Query: 305 XXXXXXXXXXXXXAANMSLLQAAAFLFVSQASCGLFLAMVFSVGHNGMEVFDKDSKPDF NMSL + A+ + QASCGL LA+VFS+GHNGM V++++KPDF
- 1110 931 GLIVHYIWQLAIPYFCNMSLFEGVAYFLMGQASCGLLLALVFSIGHNGMSVYERETKPDF Sbjct:
- Query: 365 WKLQVLSTRNVTSSLWIDWFMGGLNYQIDHHLFPMVPRHNLPALNVLVKSLCKQYDIPYH
- 1290 Sbjct: 1111WQLQVTTTRNIRASVFMDWFTGGLNYQIDHHLFPLVPRHNLPKVNVLIKSLCKEFDIPFH W+LQV +TRN+ +S+++DWF GGLNYQIDHHLFP+VPRHNLP +NVL+KSLCK++DIP+H
- Query: 425 ETGFIAGMAEVVVHLERISIEFFKEFPAM 453
  - ETGF G+ EVV HL IS EF EFPAM
- Sbjct: 1291ETGFWEGIYEVVDHLADISKEFITEFPAM 1377